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A STUDY OF DREYER'S AGGLUTINATION TECHNIC IN THE ARMY

ARNOLD G. EGGERTH

From the Base Laboratory, Hospital Center, Allerey, Saone et Loire, France

In the diagnosis of enteric fever in civil life, the physician has three laboratory methods by which he may confirm the clinical diagnosis: (1) blood culture, (2) stool and urine culture, and (3) agglutination tests with the serum. The last test, because of its simplicity and availability, is the one most generally employed. The cultural tests have come a little more into vogue the past few years, but still are considered to be tests of necessity rather than of choice. In the Army, where the triple typhoid prophylaxis is universally administered, the agglutination reaction has been found unreliable, and laboratory methods for the most part have been limited to cultures of blood, feces and urine.

In special investigations¹ as high as 66% of positive results have been obtained in blood cultures of cases of enteric fever. It seems, however, to be the general experience that such high percentages are rarely reached.² This is especially true of army hospitals in the field, where patients may not be received until the most favorable time for securing a positive blood culture has passed. It is possible, also, that, due to the more rapid and abundant formation of antibodies, the organisms do not remain in the blood for as long a period as in uninoculated individuals.²

Stool and urine cultures have not been found very satisfactory. They are of little value in early diagnosis, and to find the organism is difficult unless it is present in relatively large numbers.

Tables 1-5 describe the experience of this laboratory with these methods. They cover the period from Oct. 2, 1918, to Feb. 11, 1919. During this period the number of patients at the Center rose from 7,000 to 17,000 and then fell to 600. The great majority of the cultures were made by Lieutenant Bigelow, who had charge of the work up to Dec. 12, 1918.

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¹ Coleman and Buxton: *Am. Jour. Med. Sc.*, 1907, 133, p. 896.

² Krumbhaar and Smith: *Jour. Infect. Dis.*, 1918, 23, p. 126.

TABLE 1
SUMMARY OF CULTURES MADE

Total number of blood cultures.....	65	
Total number of stool cultures.....	109	
Total number of urine cultures.....	27	
Total		201
Total number of patients from whom blood, feces, and urine were cultured....	12	
Total number of patients from whom blood and feces alone were cultured.....	15	
Total number of patients from whom blood alone was cultured.....	25	
Total number of patients from whom feces, or feces and urine alone, were cultured	64	
Total number of patients cultured.....		116

TABLE 2
BLOOD CULTURES

		Typhoid	Para- typhoid A	Para- typhoid B	Total Positive Results
Individuals cultured once.....	43	9	1	2	12
Individuals cultured twice.....	6	0	0	0	0
Individuals cultured 3 times.....	2	0	0	0	0
Individuals cultured 4 times.....	1	0	0	0	0
Total individuals cultured.....	52				12
Total blood cultures made.....					65

TABLE 3
STOOL CULTURES

Individuals cultured once.....	78	Typhoid.....	5
Individuals cultured twice.....	10	Paratyphoid A.....	0
Individuals cultured 3 times.....	2	Paratyphoid B.....	0
Individuals cultured 4 times.....	1		
Total individuals* cultured.....	91	Total positive results...	5
Total cultures made.....			109

One of the five individuals with positive stool cultures had a positive urine and a negative blood culture; one had a positive blood culture; for the remaining three, no blood or urine cultures were made.

TABLE 4
URINE CULTURES

Individuals cultured once (positives, typhoid 2).....	9
Individuals cultured twice.....	6
Individuals cultured 3 times.....	2
Total individuals cultured.....	17
Total cultures made.....	27

One of the two positive cases gave negative stool and blood cultures; one gave a positive stool and negative blood culture.

TABLE 5
CASES GIVING POSITIVE RESULTS

Blood culture alone.....	11
Both blood and stool cultures.....	1
Stool culture alone.....	3
Both stool and urine cultures.....	1
Urine culture alone.....	1
Total.....	17
Total number of individuals cultured.....	116

Krumbhaar and Smith² give the average percentage of positive blood cultures in inoculated typhoid suspects as 11%. The percentage of positives found in this laboratory is considerably higher—23.1%—and approaches the proportion given by them for uninoculated patients (22%). The percentage of positive stools found in this laboratory was low.

It must be pointed out that many ward surgeons do not appreciate the necessity for an early blood culture. In several instances, cultures were not made until the patient was convalescent, and when the report was negative the surgeons were disappointed in the laboratory for being unable to find the organism. It is worthy of note that in no case where the first blood culture was negative, did a second or third one give a positive result. Too many stools were submitted for examination as compared with blood cultures.

There were, however, a number of cases that gave definite symptoms of typhoid fever, but in which early and repeated blood cultures were negative and in which nothing could be found in the feces or the urine. The clinician was convinced that he had a case of enteric fever, but regulations for a long time prevented him from putting that diagnosis in writing without a positive report from the laboratory. It was felt by Captain Barron, the laboratory officer of this Center, that some other means of laboratory diagnosis should be invoked; under his direction I undertook the investigation here described.

The method that bears the name of the "Dreyer technic" consists, briefly, of a series of agglutinations with the patient's serum at intervals of 4-8 days, and under conditions as standard as they can be made. The results of the tests may then be plotted in a curve. A rise in the agglutination curve for one of the organisms (which is later followed by a fall) is considered as diagnostic if the curves for the other two organisms maintain the same level or fall.

Technic.—At least 2 cc of blood was drawn with a syringe from the median basilic vein and allowed to clot. One-half cc of the clear expressed serum was taken and diluted with salt solution as desired. Further graduated dilutions were made in test tubes in the usual manner.

As the Oxford "standard agglutinable strains" of killed organisms were not available, emulsions of 24-hour agar cultures of typhoid, and paratyphoid A and B were used—the same strains being used in the entire investigation. Two controls were made for each organism: one with salt solution and one with a 1:1,000 dilution of homologous stock serum. All tubes were incubated at 37 C. for 2 hours and read, using a small hand lens in reading the doubtful ones. They were then allowed to stand at room temperature over night and checked again the following morning.

A series of tests was made on 30 healthy persons that had received the triple typhoid inoculation to determine the normal titer of the serum of such individuals. For some of these, a series of two or more agglutination tests were done at intervals from 2-20 days to determine the normal variations, if any.

TABLE 6
AGGLUTINATION TITERS OF CONTROLS

No.	Date of Last Inoculation	Period Elapsed	Date at Which Serum Was Taken	Titers		
				Typhoid	Para-typhoid A	Para-typhoid B
1	April, 1918	9 mo.	1/ 9/19	+1:20	-1:20	-1:20
2	April, 1918	9 mo.	1/11/19	-1:20	-1:20	-1:20
			1/14/19	+1:20	-1:20	-1:20
			1/19/19	-1:20	-1:20	-1:20
3	July, 1918	6 mo.	2/ 7/19	+1:20	+1:20	+1:20
4	Mar. 1918	10 mo.	1/ 9/19	+1:20	+1:40	+1:20
			1/14/19	+1:20	+1:20	-1:20
5	Jan. 1918	12 mo.	1/24/19	+1:40	+1:40	+1:20
			1/17/19	+1:40	-1:20	-1:20
6	Aug. 1918	5 mo.	1/22/19	+1:80	+1:20	+1:40
			1/19/19	+1:20	-1:20	-1:20
7	April, 1918	9 mo.	2/ 7/19	+1:20	+1:20	-1:20
			1/20/19	+1:160	+1:40	+1:20
			1/26/19	+1:80	+1:40	+1:40
8	June, 1918	7 mo.	2/ 7/19	+1:80	+1:40	+1:40
			1/20/19	+1:40	+1:20	+1:20
			2/ 1/19	+1:20	-1:20	-1:20
9	Nov. 1917	14 mo.	1/20/19	+1:40	+1:40	+1:40
10	Oct. 1917	15 mo.	1/20/19	+1:40	+1:20	+1:20
11	Mar. 1917	22 mo.	1/20/19	+1:160	+1:20	-1:20
12	April, 1918	9 mo.	1/21/19	+1:320	+1:160	+1:80
			2/ 1/19	+1:320	+1:40	+1:80
13	May, 1918	8 mo.	1/21/19	+1:20	+1:20	+1:80
14	Jan. 1918	12 mo.	1/21/19	+1:80	+1:80	+1:20
15	Dec. 1917	13 mo.	1/22/19	+1:20	+1:40	+1:40
16	Febr. 1917	23 mo.	1/22/19	+1:160	+1:40	+1:20
17	May, 1918	8 mo.	1/23/19	+1:120	-1:20	-1:20
18	April, 1918	9 mo.	1/25/19	+1:40	+1:80	+1:20
19	Jan. 1918	12 mo.	1/26/19	+1:40	-1:20	-1:20
20	Mar. 1918	10 mo.	1/26/19	+1:40	+1:40	+1:40
21	Febr. 1917	23 mo.	1/26/19	-1:20	-1:20	+1:40
22	Dec. 1917	13 mo.	1/26/19	+1:20	-1:20	-1:20
23	Jan. 1918	12 mo.	1/26/19	+1:20	-1:20	-1:20
24	Aug. 1917	17 mo.	1/26/19	+1:160	+1:40	+1:80
25	Nov. 1917	14 mo.	1/26/19	+1:20	+1:20	+1:20
26	Sept. 1917	16 mo.	1/26/19	+1:80	+1:20	+1:40
27	Jan. 1918	12 mo.	2/ 1/19	+1:80	+1:20	+1:20
28	Aug. 1917	17 mo.	2/ 1/19	+1:40	+1:20	+1:20
29	July, 1917	18 mo.	2/ 1/19	+1:80	+1:20	+1:20
30	Sept. 1917	16 mo.	2/ 7/19	+1:40	+1:20	+1:20
Average.....				+1:60	+1:26	+1:23

The plus sign in the table means the greatest dilution at which agglutination occurred. Results expressed "-1:20"—mean that no agglutination occurred at this dilution—the lowest dilution used. The averages given at the foot of the table were obtained by counting all of those negatives at -1:20 dilution as zero; when two or more agglutination tests were made of the same individual the average titer was counted. Whether the higher average for typhoid over the other two organisms signifies an actual preponderance of typhoid antibodies, or whether it means that the typhoid strain used was more agglutinable than the paratyphoids, is difficult to say. Garrow⁸ found an average typhoid agglutination titer of 1:40 seven months after inoculation, and of 1:20 sixteen months after.

⁸ Jour. Path. and Bacteriol., 1909, 13, p. 331.

It will be observed that 5 of the 30 individuals tested had a titer of 1:60 or more; only one gave a typhoid agglutination of 1:320 which was found on two different occasions. This man's stool was plated out to see if he might be a carrier, but it was found negative. It will also be noticed that when two or more tests were made on the serum of the same person, it was a common experience to find small variations. In all but two cases the variation was only small, and may therefore be accounted for as due to experimental error. In Nos. 5 and 12, however, there are greater variations, and whether these were true fluctuations or experimental errors of a grosser kind, would require the experience of other investigators to decide. But in no cases were variations found as extensive as in true cases of enteric fever.

Only one vaccination experiment was attempted, as this is a phase of the subject that has been extensively worked out by others.^{3, 4} The individual of this one experiment had received five triple typhoid inoculations during the months of March and April, 1918. Thus nine months had elapsed since the last inoculations. Two preliminary titrations of the serum were first made as follows:

Date	Titers		
	Typhoid Bacilli	Para- typhoid A	Para- typhoid B
1/ 9/19.....	+1:20	-1:20	-1:20
1/11/19.....	-1:20	-1:20	-1:20

On 1/11/19 he received subcutaneously 200,000,000 typhoid organisms killed with 0.5% phenol, but not heated. Further titrations were made as follows:

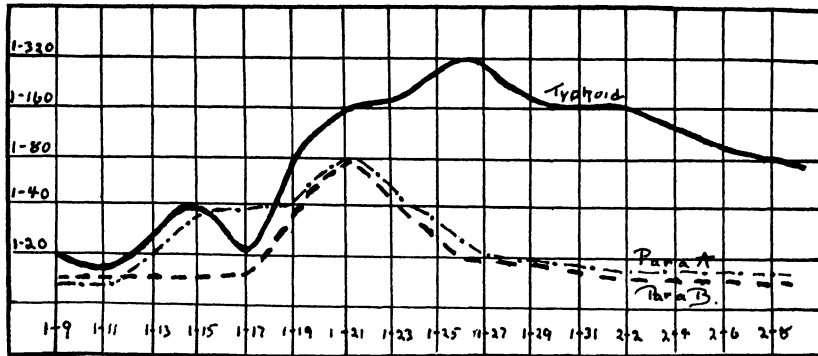
Date	Titers		
	Typhoid Bacilli	Para- typhoid A	Para- typhoid B
1/14/19.....	+1:40	-1:20	+1:40
1/17/19.....	+1:20	-1:20	+1:40
1/19/19.....	+1:80	+1:40	+1:40
1/21/19.....	+1:160	+1:80	+1:80

On 1/21/19 another injection of 200,000,000 killed typhoid organisms was given.

Date	Titers		
	Typhoid Bacilli	Para- typhoid A	Para- typhoid B
1/23/19.....	+1:160	+1:40	+1:40
1/26/19.....	+1:320	+1:20	+1:20
1/30/19.....	+1:160	+1:20	+1:20
2/ 2/19.....	+1:160	-1:20	+1:20
2/ 9/19.....	+1:80	-1:20	+1:20

⁴ Fennel: Jour. Am. Med. Assn., 1918, 70, p. 590.

Plotting these data gives the following curve:



Only two small inoculations were made. The curve, while small, corroborates the results of others, and reproduces what was actually found in clinical cases of enteric fever. While the paratyphoid curves showed an early rise, there was also a quick falling off, even while the typhoid curve was still rising. Such a curve obtained in a clinical case should be characteristic enough to permit of a positive diagnosis.

In the course of this investigation (which was not begun until Dec. 16, 1918, when patients were already being evacuated in large numbers) titrations were made of the serums of 13 patients. In the blood of 5 of these, an organism of the typhoid group had been found. These 5 will be discussed first.

1. Triple typhoid prophylaxis in June, 1917. Date of beginning of illness was not ascertained. A blood culture Nov. 25, 1918, gave paratyphoid B bacilli. Serum for agglutination obtained Dec. 20, when the patient was already out of bed and awaiting evacuation, gave titers as follows: Typhoid bacilli, —1:50; paratyphoid A, —1:50; paratyphoid B, +1:50. The paratyphoid B titer was low. This case, being tested too late, is of little value.

2. Triple typhoid prophylaxis April, 1918. Admitted to hospital Nov. 15, 1918. Typhoid bacilli in blood culture Nov. 25. First agglutination Dec. 24; patient still in bed: Typhoid bacilli, +1:400; paratyphoid A, —1:100; paratyphoid B, —1:100. The importance of testing lower dilutions was not realized at this time. Second agglutination Jan. 11, 1919: Typhoid bacilli, +1:160; paratyphoid A, —1:20; paratyphoid B, +1:40. The first test in this case was not done until a month after the positive blood culture; an earlier test would undoubtedly have given a much higher reading; though even at this late date considerable typhoid agglutinin was still present.

3. Triple typhoid prophylaxis in April, 1917. Taken ill Dec. 23, 1918. Paratyphoid A found in a blood culture Dec. 28. Agglutination tests as follows:

Date	Titers		
	Typhoid Bacilli	Para-typhoid A	Para-typhoid B
12/28/18.....	+1:20	+1:6400	+1:20
1/ 3/19.....	+1:40	+1:3200	+1:40
1/14/19.....	+1:40	+1:1280	+1:20
1/23/19.....	+1:40	+1:1280	+1:20
2/10/19.....	+1:20	+1:640	+1:20

This case shows early rise in the paratyphoid A curve, the first agglutination being the maximum found. The patient was never very ill, and enjoyed an uneventful convalescence. It is interesting to note that altho the titer at the time of blood culture was 1:6400, it was only one-fifth that amount less than a month later, and only one-tenth after 6 weeks.

4. Triple typhoid prophylaxis August, 1917. Admitted Nov. 26, 1918. B. typhosus found in stool Dec. 2; blood culture negative. No agglutination done at this time. On the night of Jan. 22, 1919, relapse; blood culture Jan. 25 positive for typhoid bacillus. Agglutination titers: Typhoid bacilli, +1:80; paratyphoid A, +1:20; paratyphoid B, —1:20. The clinical prognosis at that time very grave; however, he rallied in a few days and is progressing favorably. Feb. 2, agglutination: typhoid bacilli, +1:640; paratyphoid A, +1:20; paratyphoid B, —1:20.

This case differs much from the preceding. In spite of the recent attack of typhoid, the agglutinating titer was low at the time of blood culture, but 8 days later it had risen from +1:80 to +1:640. The parallelism between the low initial titer and the unfavorable clinical prognosis, and then between the rising titer and the favorable prognosis, may be significant.

5. Triple typhoid prophylaxis in June, 1918; admitted Feb. 5, 1919. A blood culture Feb. 10, gave typhoid bacilli. Only one agglutination test, with this result: typhoid bacilli, +1:5120; paratyphoid A, +1:40; paratyphoid B, +1:40. This case is of the same type as case 3. At this date (Feb. 20) the patient seems to be on the road to recovery.

In addition to these five cases, agglutination tests were made in cases clinically diagnosed as typhoid, but in which blood and stool cultures were negative. Unfortunately, most of the tests were so late in the course of the disease that very little information was obtained.

Date on Which Serum Was Taken	Date of Inoculation	Date of Admission	Titers		
			Typhoid Bacilli	Para-typhoid A	Para-typhoid B
1. 12/16/18	Aug., 1918	Not ascertained	—1:100	—1:100	—1:100
2. 12/16/18	Aug., 1918	Not ascertained	+1:200	—1:100	+1:3200
3. 12/20/18	Not ascertained	Not ascertained	—1:100	—1:100	+1:3200
12/28/18			+1:80	—1:20	+1:6400
4. 12/20/18	April, 1918	Not ascertained	—1:100	—1:100	—1:100
5. 12/21/18	March, 1918	Nov. 15, 1918	—1:100	+1:400	+1:400
1/14/19			+1:80	+1:320	+1:160
6. 12/24/18	May, 1918	Nov. 20, 1918	+1:200	—1:100	—1:100
1/17/19			+1:320	—1:20	+1:20
7. 12/24/18	July, 1917	Dec. 6, 1918	+1:400	+1:400	—1:100
1/11/19			+1:320	+1:320	+1:20
8. 12/24/18	Sept., 1917	Nov. 1, 1918	+1:100	—1:100	—1:100
1/14/19			+1:160	—1:20	—1:20

As said, the important early tests were not made in these cases, which takes from the results most of their value. Cases 2 and 3, are of great interest, however, in that they show so high a titer for paratyphoid B, though both cases were in the later stages of convalescence. Cases 5 and 7 show a high titer for each of two organisms—a phenomenon that has been observed by others.² It is necessary to bear in mind that the effect of other infections, such as influenza or pneumonia, on the typhoid group agglutinins, has not been studied in this investigation. While it is not believed that “non-specific stimu-

lation" of the agglutination titer would be great enough to cause confusion, yet it would be well to study the typhoid agglutinins in such infections, to guard against a possible fallacy.

CONCLUSIONS

Another means of diagnosis of enteric fever, to supplement blood, stool and urine cultures, is highly desirable.

The agglutinating titer of the serum of healthy individuals inoculated with the triple typhoid vaccine six months or more before rarely runs higher than 1:200.

A high agglutinating titer for one of the organisms of the typhoid group permits of a positive diagnosis. Just how high this titer must be depends on the time that has elapsed since the prophylactic inoculation. A positive agglutination at 1:1000 in individuals that give a clinical picture of typhoid would seem to be of diagnostic value, if they have been inoculated for more than six months. Further tests should be made at intervals of 5 to 8 days.

In all cases in which a marked rise in the agglutinating titer for one of the organisms can be followed, while the agglutinins for the other two remain constant or diminish, a positive diagnosis may be made. A slight rise is of no diagnostic value.

The cases studied suggest strongly that information as to the prognosis may be obtained from the agglutinin curve. Thus it may be that a patient presenting a high titer will progress favorably—barring complications—while cases in which the titer is low may later develop relapse. It would be of great value to determine if a relapse could be foretold by finding low titer in cases that progress unfavorably.

Early blood culture should not be omitted. The Dreyer method should be used when the blood culture is negative, in a clinically suggestive case.

The following procedures are suggested in the order of their importance:

A. For inoculated patients:

1. Blood culture.
2. Dreyer's technic.
3. Stool and urine cultures.

B. For uninoculated patients:

1. Agglutination test and blood culture.
2. Stool and urine culture.
3. Dreyer's technic for prognosis.